



# Combined Effects of Simulated Microgravity and Radiation Exposure on Osteoclast Cell Fusion

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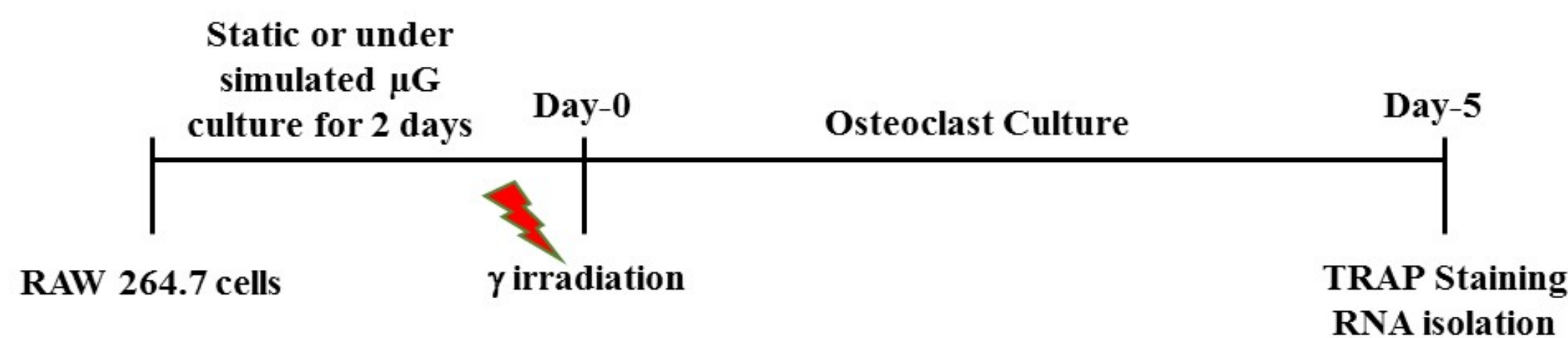
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## Introduction

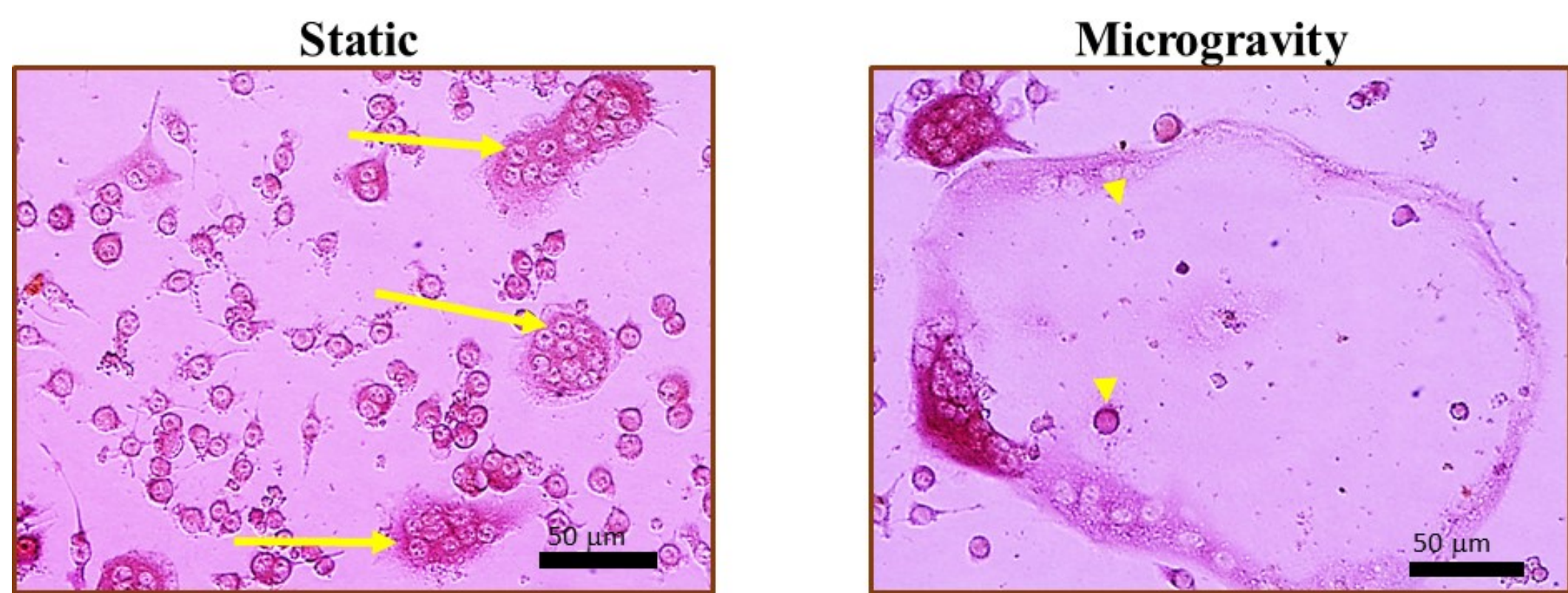
The loss of bone mass and alteration in bone physiology during space flight are one of the major health risks for astronauts. Although the lack of weight bearing in microgravity is considered a risk factor for bone loss and possible osteoporosis, organisms living in space are also exposed to cosmic radiation and other environmental stress factors. As such, it is still unclear as to whether and by how much radiation exposure contributes to bone loss during space travel, and whether the effects of microgravity and radiation exposure are additive or synergistic. Bone is continuously renewed through the resorption of old bone by osteoclast cells and the formation of new bone by osteoblast cells. In this study, we investigated the combined effects of microgravity and radiation by evaluating the maturation of a hematopoietic cell line to mature osteoclasts. RAW 264.7 monocyte/macrophage cells were cultured in rotating wall vessels that simulate microgravity on the ground. Cells under static 1g or simulated microgravity were exposed to  $\gamma$  rays of varying doses, and then cultured in receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) for the formation of osteoclast giant multinucleated cells (GMCs) and for gene expression analysis. Results of the study showed that radiation alone at doses as low as 0.1 Gy may stimulate osteoclast cell fusion as assessed by GMCs and the expression of signature genes such as tartrate resistant acid phosphatase (*Trap*) and dendritic cell-specific transmembrane protein (*Dcstamp*). However, osteoclast cell fusion decreased for doses greater than 0.5 Gy. In comparison to radiation exposure, simulated microgravity induced higher levels of cell fusion, and the effects of these two environmental factors appeared additive. Interestingly, the microgravity effect on osteoclast stimulatory transmembrane protein (*Ocstamp*) and *Dcstamp* expressions was significantly higher than the radiation effect, suggesting that radiation may not increase the synthesis of adhesion molecules as much as microgravity.

## Materials and Methods

- RAW 264.7 monocyte/macrophage cells were cultured in rotating wall vessels that simulate microgravity on the ground.
- Cells under static 1g or simulated microgravity were exposed to  $\gamma$  rays of varying doses.
- Cells were cultured in receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) for the formation of osteoclast giant multinucleated cells (GMCs) and for gene expression analysis.

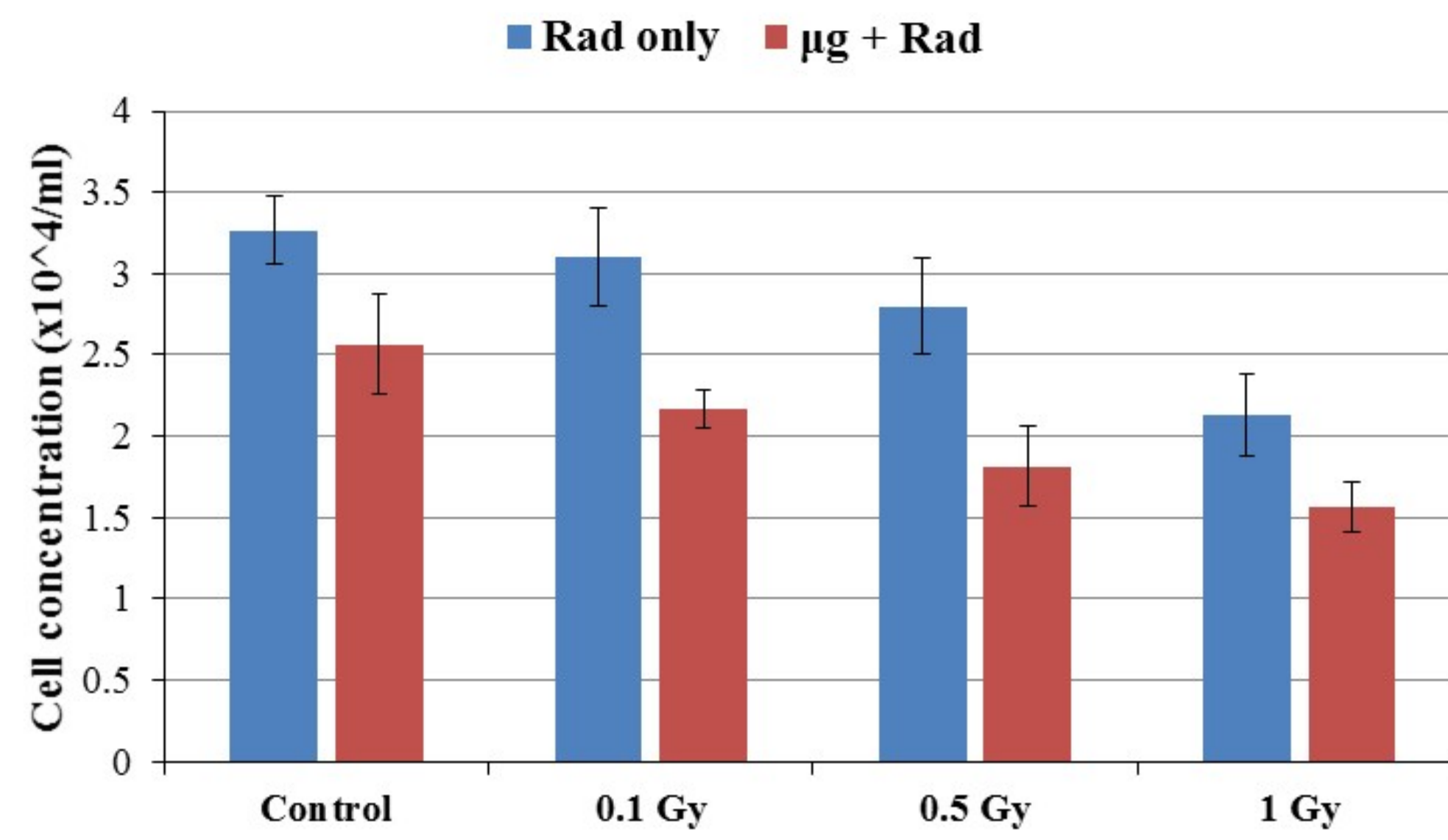


**Figure 1.** Timeline of the experiment. RAW 264.7 cells were cultured in static or under simulated microgravity for 48 h before exposed to varying doses of  $\gamma$  rays. Cells were then cultured in the presence of RANKL for 5 days for quantification of osteoclast differentiation and for gene expression analysis.

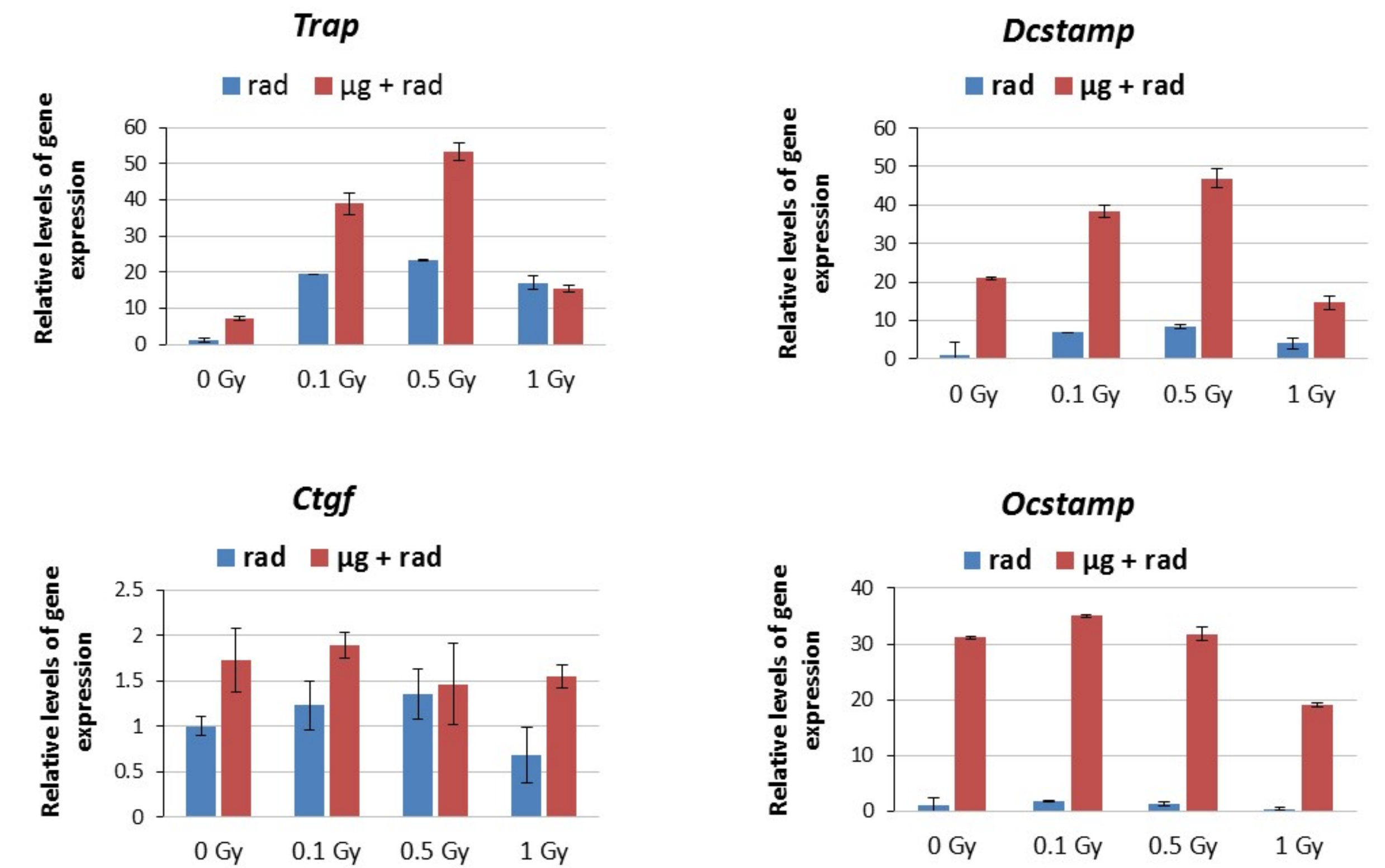


**Figure 2.** Multinucleated osteoclasts (pointed by arrows) formed in cells after a 5-day osteoclast culture in RANKL. Cells were maintained in static conditions or under simulated microgravity for 48 h immediately prior to osteoclast culture.

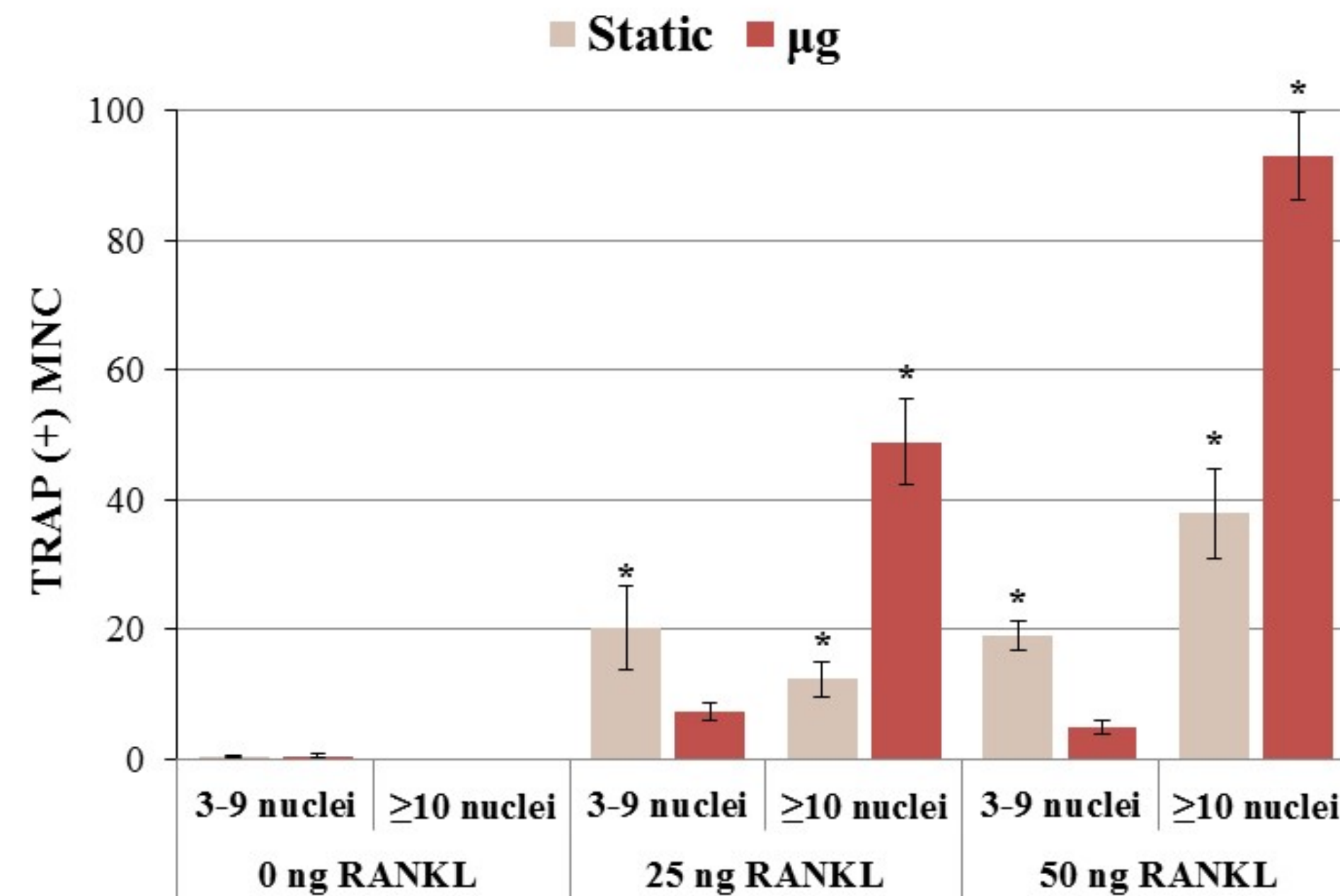
## Results



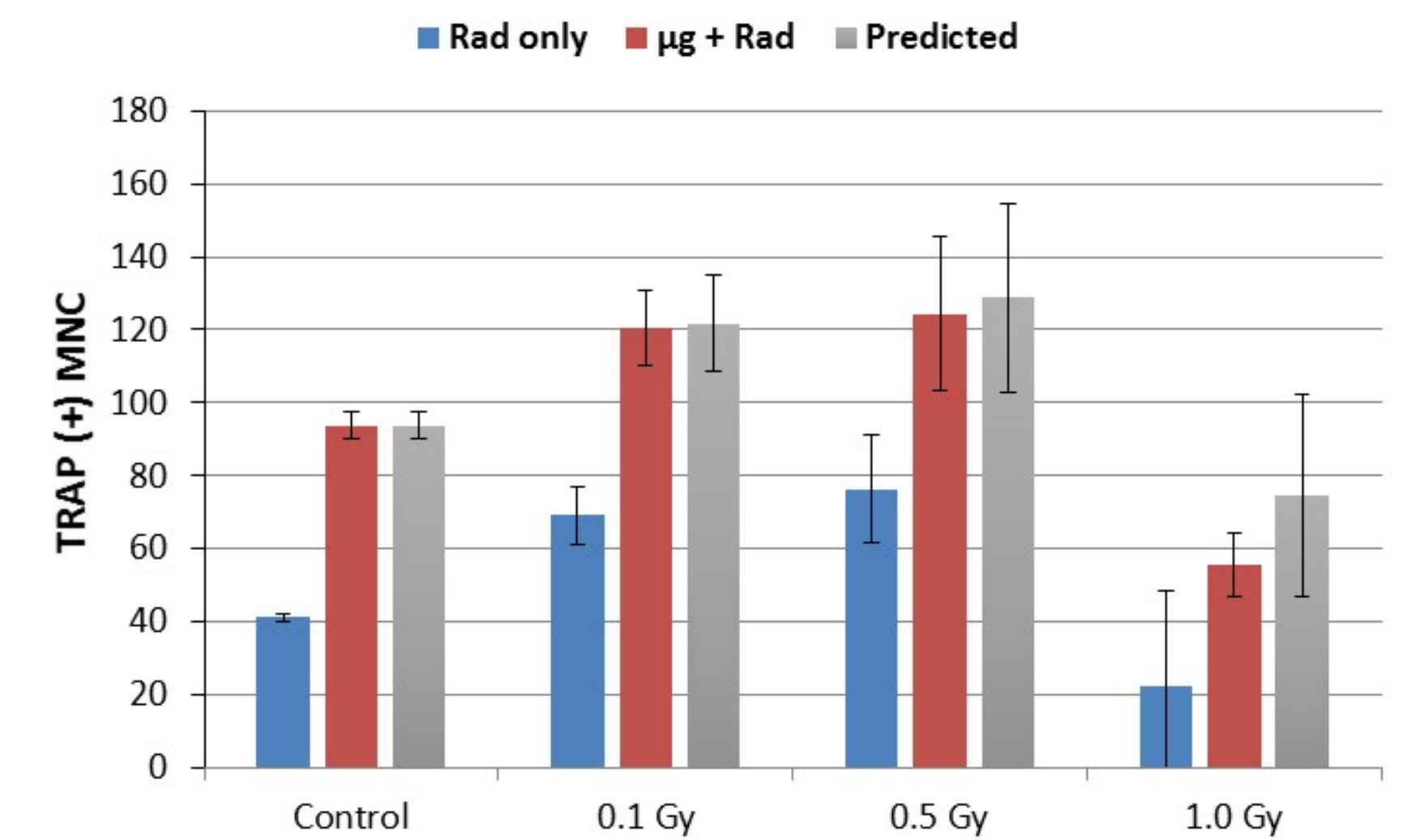
**Figure 3:** Human mammary epithelial cells (M10) had higher fraction of chromosomal aberration after Fe ion radiation at both early and late time points, compared to lymphocytes, while lymphocytes and mammary epithelial cells showed similar degree of chromosomal aberration after proton exposure at early time points. Data are percent of aberrations in total counted metaphase spreads.



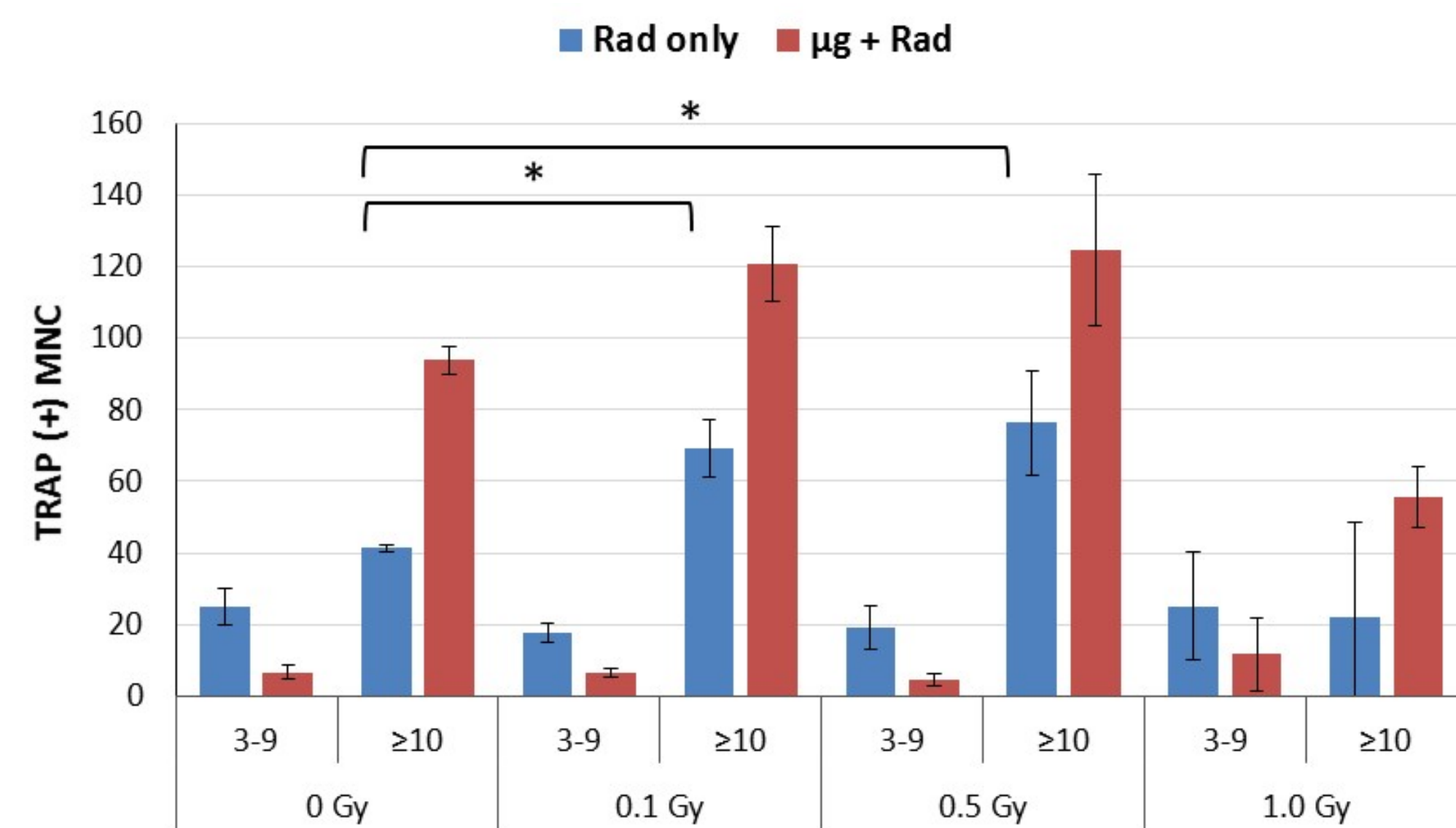
**Figure 6.** Expression of *Trap*, *Ocstamp*, *Dcstamp*, and *Ctgf* genes in response to different doses of gamma irradiation under the static (blue bars) and simulated microgravity (red bars) conditions. The increase in gene expression peaked at doses of 0.1–0.5 Gy. For all genes, except for *Ctgf*, expression was significantly higher in radiation + microgravity compared to radiation alone (two-way ANOVA of triplicates per each condition and dose;  $p = 0.0001$ ).



**Figure 4.** RANKL-dependent multinucleated cells formation under static and simulate microgravity. multinucleated cells (MNCs) with  $\geq 10$  nuclei significantly increases in microgravity compared to static condition (two-way ANOVA:  $p < 0.0001$ ). The number of MNCs containing  $\geq 10$  nuclei increased significantly with increasing RANKL concentration in both static (one-way ANOVA:  $p = 0.0002$ ) and microgravity (one-way ANOVA:  $p = 0.0001$ ) conditions. Stars mean statistical significance compared to the corresponding control using Dunnett's multiple comparison test (\*\*\*)  $p < 0.005$ .



**Figure 7.** Osteoclast fusion prediction. Blue and red bars represent MNCs containing  $\geq 10$  nuclei analyzed in Figure 3 for radiation alone and combined radiation and microgravity exposures, respectively. Microgravity alone induced about 53 GMCs ( $=94 - 41$ ) per dish in comparison to the background (0 Gy). The predicted number based on additive effects of radiation and microgravity (gray bars) is then the sum of this number and the number of GMCs after irradiation alone. For all three doses of 0.1, 0.5, and 1 Gy, the combined effects (red bars) agreed well with the prediction calculated based on the additive effects (gray bars).



**Figure 5.** Induction of osteoclast fusion after radiation exposure static (blue bars) and under microgravity (red bars). 0.1 Gy and 0.5 Gy radiation significantly ( $* p < 0.05$   $t$ -test compared to 0 Gy) stimulated osteoclast fusion, but not 1 Gy. The number of multinucleated cells containing  $\geq 10$  nuclei increased significantly in radiation + simulated microgravity conditions compared with radiation alone (two-way ANOVA:  $p = 0.0001$ ). Error bars mean SD from three independent experiments.

## Conclusions

- Radiation alone at doses as low as 0.1 Gy may stimulate osteoclast cell fusion as assessed by GMCs and the expression of signature genes such as tartrate resistant acid phosphatase (*Trap*) and dendritic cell-specific transmembrane protein (*Dcstamp*).
- Osteoclast cell fusion decreased for doses greater than 0.5 Gy.
- Simulated microgravity induced higher levels of cell fusion, and the effects of these two environmental factors appeared additive.
- Microgravity effect on osteoclast stimulatory transmembrane protein (*Ocstamp*) and *Dcstamp* expressions was significantly higher than the radiation effect, suggesting that radiation may not increase the synthesis of adhesion molecules as much as microgravity.